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Identification of Primary and Secondary Measles Vaccine Failures by Measurement of Immunoglobulin G Avidity in Measles Cases during the 1997 São Paulo Epidemic

Cláudio S. Pannuti,[1]* Ricardo José Morello,[1,2] José Cássio de Moraes,[3] Suely Pires Curti,[4] Ana Maria S. Afonso,[4] Maria Cláudia Corrêa Camargo,[3] and Vanda A. U. F. de Souza[1]

Instituto de Medicina Tropical de São Paulo—LIM-HC, Departamento de Moléstias Infecciosas e Parasitárias, Faculdade de Medicina[1] and Departamento de Microbiologia, Instituto de Ciências Biomédicas,[2] Universidade de São Paulo, Secretaria de Estado da Saúde de São Paulo, Centro de Vigilância Epidemiológica Professor Alexandre Vranjac,[3] and Laboratório de Vírus Exantemáticos, Instituto Adolfo Lutz,[4] São Paulo, Brazil

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Despite almost universal use of measles vaccines in recent decades, epidemics of the disease continue to occur. Understanding the role of primary vaccine failure (failure to seroconvert after vaccination) and secondary vaccine failures (waning immunity after seroconversion) in measles epidemics is important for the evaluation of measles control programs in developing countries. After a measles epidemic in São Paulo, Brazil, 159 cases previously confirmed by detection of specific immunoglobulin M (IgM) antibodies were tested for IgG avidity, and a secondary immune response, defined by an IgG avidity index of at least 30%, was established in 30 of 159 (18.9%) patients. Among the 159 patients, 107 (67.3%) had not been vaccinated and 52 (32.7%) had received one or more doses of measles vaccine. Of the 107 unvaccinated patients, 104 (97.2%) showed a primary immune response, defined as an IgG avidity index of less than 30%. Among the 52 patients with documented vaccination, 25 (48.1%) showed a primary immune response and 27 (51.9%) showed a secondary immune response, thereby constituting a secondary vaccine failure. Primary vaccine failure was observed in 13 of 13 patients vaccinated prior to 1 year of age and in 43.5 and 12.5%, respectively, of patients receiving one or two doses after their first birthdays. These results provide evidence that measurement of IgG avidity can be used to distinguish between primary and secondary vaccine failures in vaccinated patients with measles; the method can also be a useful tool for the evaluation of measles control programs.

Despite almost universal use of measles vaccines in recent decades, epidemics of the disease continue to occur. In 1997, 20,186 laboratory-confirmed cases of measles were reported in an epidemic occurring in the State of São Paulo, Brazil. Of the 19,322 confirmed measles cases in which the age of the patient was known, 9,938 (51%) occurred in persons aged 20 to 29 years (6). A residential survey conducted after the epidemic to determine predictors of measles occurrence in the county of São Paulo showed that 31.9% of cases occurred in persons who had received one or more doses of the vaccine (4).

Most cases of measles in vaccinated persons occur in the subset of individuals who did not undergo serological conversion after vaccination. This is known as primary vaccine failure (12). The frequency of primary vaccine failure is variable and has been shown to be a function of age at the time of vaccination, the number of doses, the immunogenicity of the strain of the virus used to manufacture the vaccine, and the geographic region (3, 20, 25). Secondary vaccine failure is defined as the occurrence of measles in persons in whom postvaccination serologic conversion has been documented (19, 20, 25, 28).

The assays currently available for detecting anti-measles immunoglobulin M (IgM) antibodies show a high sensitivity for measles diagnosis (10, 11, 15, 17), and 100% of persons with measles test positive by IgM capture enzyme immunoassay (EIA) when samples are collected within 4 to 11 days after the onset of rash (15). Improved assay sensitivity for IgM detection, however, resulted in additional difficulties in distinguishing between primary and secondary vaccine failure in measles patients who had been vaccinated, because the IgM capture EIA result may be positive for some patients with secondary vaccine failure (10, 15, 16). Approaches used to deal with this problem include the determination of the IgM/IgG ratio (7, 10) and differences in antibody titers and times to seroconversion (16).

The test for assessment of IgG antibody avidity is a reliable tool for differentiating between the immune response occurring in immunologically naive patients (primary immune response) and the immune response that occurs in patients with a preexisting B-cell memory (secondary immune response) (13). The test uses the fact that, in primary infection, the specific IgG antibody response begins with IgG antibodies that bind weakly with antigens (low avidity), which gradually evolve to become high-avidity antibodies (i.e., antibodies that have a stronger binding with antigens). In the secondary infection, the rapid antibody response is characterized by the production of high-avidity antibodies (22).

The IgG antibody avidity test has been shown to be very useful for diagnosing recent primary rubella (8, 14), toxoplasmosis (21), and cytomegalovirus infection (2, 27) in pregnant women; for distinguishing primary hepatitis C virus infection

* Corresponding author. Mailing address: Laboratório de Virologia (LIMHC-52), Instituto de Medicina Tropical de São Paulo, Av. Dr. Enéas de Carvalho Aguiar, 470, 05403-000 São Paulo (SP), Brazil. Phone: 55-11-3066-7012. Fax: 55-11-3063-2659. E-mail: cpannuti@usp.br.

from chronic or past hepatitis C virus infection (18); and for serodiagnosis of many other acute viral diseases (1, 13, 29). Regarding measles infection, the IgG avidity test has been used for estimating the efficacy of measles vaccines (31) and for identifying secondary vaccine failures (25). More recently, it has been proposed that the IgG avidity test for measles may have a potential role in studies of measles vaccine-induced immunity for use as a more effective global measles elimination strategy (26). The aim of the present study was to identify primary and secondary vaccine failures by measurement of IgG avidity in IgM-confirmed measles cases that occurred during the 1997 São Paulo epidemic.

MATERIALS AND METHODS

Population. The present study was carried out by using the serum samples and the database of a previous investigation, which was undertaken to identify predictors of measles occurrence in children and young adults (<30 years of age) who constituted more than 90% of the measles cases during the 1997 epidemic in São Paulo County (4). In that study, patients' data, including the measles vaccination status as indicated on the official vaccination cards, were obtained from questionnaires completed during a residential survey carried out from November 1997 to March 1998. This study was approved by the Research Ethics Committee of Instituto Adolfo Lutz, São Paulo, Brazil.

Criteria for inclusion in study. The measles cases chosen fulfilled all the following criteria: (i) presence of a rash that met the clinical measles case definition of the Centers for Disease Control and Prevention, (ii) laboratory confirmation of measles by detection of specific IgM antibodies in a sample collected within 30 days after the onset of rash, (iii) patients younger than 30 years, (iv) indication of vaccination status on the official vaccination card, and (v) no vaccination for measles 5 to 21 days prior to the onset of rash.

Serum samples. The serum samples for the IgG avidity test were chosen from the serum bank, which is maintained at −20°C at the Laboratory of Exanthematic Viruses of the Instituto Adolfo Lutz.

Detection of IgM antibodies. Detection of IgM antibodies was performed by employing a commercial, indirect EIA (Enzygnost-Anti-Measles Virus IgM; Dade Behring, Marburg, Germany) or by an IgM capture EIA (17).

Detection of IgG antibodies. Measles IgG antibodies were detected by an in-house enzyme-linked immunosorbent assay as previously described (30). Anti-measles IgG antibody titers, expressed as milli-international units per milliliter, were estimated with serial dilutions of a reference serum provided by the National Institute for Biological Standards and Control (Hertford, England) that were included in each run of our enzyme-linked immunosorbent assay. The titers were calculated from the regression line by using Microsoft Excel. Samples with titers lower than 100 mIU/ml were considered negative.

Avidity of anti-measles IgG antibodies. IgG avidity was estimated as previously described (31), with some modifications. This technique is based on the dissociation of low-avidity antibodies upon incubation with urea (14). Briefly, microplates (Nunc-Immuno Plates, Polysorp; Nalge Nunc International) were coated with measles antigen extracted from Vero cells infected with the Toyoshima strain of measles virus and with control antigens obtained from uninfected Vero cells. The microplates were incubated for 1 h at 37°C with 200 μl of blocking solution, which consisted of phosphate-buffered saline (PBS) (pH 7.2) plus 0.05% (vol/vol) Tween 20 (PBST) and 5% (wt/vol) skimmed milk. The blocking solution was then discarded, and 50 μl of serum diluted 1:100 was added in duplicate (two wells containing viral antigen and two wells containing control antigen).

After incubation for 1 h at 37°C, the plates were washed twice with PBST and half the wells were soaked for 5 min with 8 M urea in PBST and half with PBST without urea. After two additional washes, 50 μl of peroxidase-conjugated anti-human IgG (Gibco-BRL) was added. The plates were incubated for 40 min at 37°C and were washed four times with PBST, after which 50 μl of the substrate and chromogen solution (0.03% hydrogen peroxide and 0.4 mg of *o*-phenylenediamine per ml in 0.1 M citrate buffer [pH 5.0]) were added. After incubation in a dark chamber for 20 min at room temperature, the enzymatic reaction was stopped by the addition of 50 μl of 2.5 N H_2SO_4. The plates were read at 492 nm with a microplate spectrophotometer (Titertek Multiskan II).

The results, reported as the difference in optical density (ΔOD), were calculated by subtracting the optical density obtained for the viral antigen from that obtained for the control cells, which either were or were not treated with urea. The avidity index (AI) was calculated by using the following formula: AI (%) = [(ΔOD with urea)/(ΔOD without urea)] × 100. Samples with an AI less than 30% were considered to be of low avidity.

Definition of primary and secondary immune responses. A patient with a primary immune response was defined as a subject in whom a serum sample collected within 30 days after the onset of rash was IgM positive and IgG negative or was IgM positive with a low-AI IgG titer. Persons with titers equal to or greater than 100 mIU/ml with an AI equal to or greater than 30% were considered to have had a secondary response.

Definition of primary and secondary vaccine failure. A patient with primary vaccine failure was defined as one with a previous measles vaccination recorded on an official vaccination card and in whom a primary immune response was demonstrated serologically. A patient with secondary vaccine failure was defined as a vaccinated subject showing a secondary immune response in a serum sample collected within 30 days after the onset of rash.

Statistical analysis. Statistical analysis was performed by using EpiInfo 6, version 6.04d (Centers for Disease Control and Prevention). The rates of primary and secondary responses according to the number of vaccine doses were compared by chi-square or Fisher's exact test when appropriate.

TABLE 1. Anti-measles antibody titers and IgG avidity in 159 patients with measles, as a function of vaccination history

Vaccination history	No. of patients	No. of patients with titer <100 mIU/ml (%)	No. of patients with titer >100 mIU/ml	
			Low avidity (%)	High avidity (%)
Not vaccinated	107	87 (81.3)	17 (15.9)	3 (2.8)
Vaccinated	52	7 (13.5)	18 (34.6)	27 (51.9)
Total	159	94 (59.1)	35 (22.0)	30 (18.9)

RESULTS

The serum samples analyzed were collected up to 27 days after the onset of rash, with 90% of the samples being obtained within 5 days of rash onset. Among the 159 patients enrolled in the present study, 107 (67.3%) had not been vaccinated and 52 (32.7%) had received one or more doses of measles vaccine. Irrespective of vaccination status, a primary immune response was established serologically for 129 (81.1%) patients and a secondary immune response was observed for 30 of 159 (18.9%) patients. One hundred four (97.2%) of the 107 unvaccinated patients showed a primary serological response. Among the 52 patients who had been vaccinated, 25 (48.1%) showed a primary immune response and 27 (51.9%) showed a secondary immune response, thereby constituting a secondary vaccine failure (Table 1).

Analysis of the type of immune response according to age of vaccination and number of vaccine doses showed that 2 of 16 (12.5%) measles patients who received two doses of vaccine after 1 year of age had a primary vaccine failure. This rate was significantly lower than the primary failure rate observed in the group of patients who had received a single dose of vaccine before 1 year of age (13 of 13, 100%; $P < 0.0001$) or in the group of patients who had received a single dose after 1 year of age (10 of 23, 43.5%; $P = 0.04$, one-tailed Fisher's exact test) (Table 2).

DISCUSSION

In 1994, the Pan American Health Organization set a goal for stopping indigenous measles transmission in the Americas

TABLE 2. Type of immune response (primary and secondary) in 52 vaccinated patients with measles, as a function of vaccination schedule

Vaccination schedule (dose and age)	Total no. of patients	Primary response		Secondary response	
		n (%)	95% CI[c]	*n* (%)	95% CI
1 dose at <1 year	13	13 (100.0)	75.2–100.0	0 (0.0)	
1 dose at >1 year[a]	23	10 (43.5)	23.1–65.5	13 (56.5)	34.4–76.8
2 doses at >1 year[b]	16	2 (12.5)[d]	1.5–38.3	14 (87.5)	61.6–98.4

[a] 19 of 23 patients had received one dose of measles vaccine before 1 year of age.
[b] 13 of 16 patients had received one dose of measles vaccine before 1 year of age.
[c] 95% confidence interval.
[d] *P* was <0.05 when compared with values for patients who had received a single vaccine dose before or after 1 year of age.

by the end of 2000. In 1996, only nine cases of measles were reported in the city of São Paulo, Brazil. However, in 1997, more than 20,000 cases of laboratory-confirmed measles were reported in that city (6). This outbreak demonstrated that the limited circulation of the measles virus does not indicate the absence of risk for measles outbreaks and highlights several challenges facing those working toward the elimination of measles.

Understanding the role of primary and secondary vaccine failures in measles epidemics is important for the evaluation of measles control programs in developing countries. A high proportion of primary vaccine failures in vaccinated patients with measles can indicate, for instance, problems in vaccine handling or use of inactive vaccines due to inadequate handling. However, the introduction of enhanced diagnostic tests for IgM detection such as the IgM capture EIA, results of which may be positive for patients with secondary vaccine failure, resulted in new difficulties for differentiating between primary and secondary vaccine failures (10, 15, 16, 25).

The estimation of IgG antibody avidity is very useful in identifying primary and secondary immune responses, but there have been few reports of its use during measles outbreaks (25). The results of the present study, which show that 18.9% of measles cases confirmed by a positive IgM test exhibited a secondary immune response, provide further evidence that the presence of IgM cannot be used as a reliable indicator of a primary immune response (10, 16, 25, 26).

It is estimated that in highly vaccinated populations, 4 to 8% of measles cases in outbreaks are due to secondary vaccine failure (9, 23). In 1990, more than 27,000 measles cases were reported in the United States, 18.4% of which occurred in subjects vaccinated at least once after reaching 1 year of age. However, the rates of primary and secondary vaccine failures in those cases were not evaluated (5). Vaccination schedules differ considerably from country to country. As a consequence, vaccine failure rates may differ from country to country. Several factors, such as age at vaccination, number of doses received, and immunogenicity of the vaccine strain, may be associated with different rates of primary and secondary vaccine failure (25).

The majority of cases in the present series (107 of 159, 67.3%) occurred in unvaccinated individuals. As expected, 104 of 107 (97.2%) unvaccinated study subjects showed a primary immune response, thereby validating the information given by the IgG avidity test. However, 18.9% of the 159 measles cases analyzed for the present study were associated with a secondary immune response, suggesting that secondary vaccine failures also played an important role in the 1997 São Paulo measles epidemic. Paunio et al. (25), who also used the IgG avidity method, reported a high incidence of secondary vaccine failure during an outbreak of measles in Finland in 1998 and 1999, with rates ranging from 7 to 50% depending on age at vaccination, vaccine strain, and the number of vaccine doses given.

Analysis of the number of vaccine doses and the type of vaccine failure showed that the group of measles patients who had received two vaccine doses after 1 year of age had a rate of primary vaccine failure significantly lower than that of the group of patients who had received a single vaccine dose before 1 year of age or of the group of patients who had received only one dose after 1 year of age. The present study was not designed to evaluate all measles cases that occurred during the 1997 epidemic. However, extrapolating the rate of primary vaccine failures of the present series to the total number of cases in the 1997 measles epidemic in the city of São Paulo, we estimate that about 3,000 cases may have resulted from primary vaccine failure. Most of those cases could probably have been avoided had those patients been given two doses of measles vaccine after 1 year of age (24).

We conclude that IgG avidity can be a useful tool for evaluating primary and secondary vaccine failures during measles outbreaks.

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